

2. Title: "Isolation of Leptospires from Thailand Modes of Transmission"

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**OBJECTIVE:** The objective of this study is to evaluate by isolation techniques the role of wild or domestic animals and water in transmission of the leptospire organism.

**DESCRIPTION:** This study consists of isolation of the leptospire organism either by inoculation into weanling hamsters or by direct culture in Fletcher's media enriched with rabbit serum. Kidneys of animals are aseptically exposed and plugs obtained with a Pasteur pipette are discharged directly into tubes of Fletcher's media. Water samples collected from streams, ponds, canals, paddy fields, buffalo wallows and mud from suspected contaminated areas are inoculated intraperitoneally into weanling hamsters. Five hamsters are inoculated with each specimen and observed for 21 days for signs of illness or death. Bloods from the ventricles of those dying between the first and fourth day were cultured to determine the presence of P. pseudomallei. Those dying from the fourth to seventh day were cultured for both P. pseudomallei and leptospiral organisms. Those dying between seven and twenty one days were cultured only for leptospiral organisms. All remaining animals were sacrificed at twenty-one days and at least one from each cage was cultured for leptospirosis. Culture techniques are the same as described above for other animals.

Since minimal success was obtained in isolating organisms from areas of central and northern Thailand it was felt that information should be obtained from provinces in southern Thailand. This area forms part of the Kra peninsula and geographically approximates Malaya where numerous isolations have been reported.

**PROGRESS:** Kidneys obtained from 180 dogs at the Bangkok dog pound were cultured. Twenty-three isolates were obtained and serotyped as follows: 12 L. bataviae, 5 L. javanica, 6 L. canicola. Twenty-two isolates were obtained from rodents trapped in Bangkok and serotyped as follows: 17 L. bataviae, 5 L. javanica. Four hundred eighty-five rodents were trapped in southern Thailand from 12 provinces ranging from the Malaysian borders to Chumporn. Twenty-six isolates were obtained. These were serotyped as follows: 11 L. javanica, 8 L. bataviae, 7 L. icterohemorrhagiae. Rodents were trapped in Chiangmai, Udorn and Korat and cultured for leptospiral organisms. To date the following isolates have been obtained but are not serotyped yet:

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<u>Number Trapped</u>	<u>Location</u>	<u>Isolates</u>
187	Udorn	10
65	Chiengmai	3
32	Korat	0

Eleven rodent isolates obtained from cultures submitted by technicians of the scrub typhus project were serotyped. Ten were serotyped L. javanica and one was L. alexi.

Seven hundred thirty-nine water samples were collected at random in 12 provinces of southern Thailand. These included collections from jungle streams, canals, ponds and surface water following heavy rainfall. The samples were inoculated into weanling hamsters as described above. A high incidence of P. pseudomallei was encountered in the southern most provinces. Nineteen leptospire were obtained. Serotypes are as follows: two each of L. autumnalis, L. bataviae, L. pomona, L. grippotyphsa and one each of L. ecterohemorrhagiae, L. malaya, L. australis, L. zanoni, L. cynopteri, L. wolffi, L. djaziman, L. butembo, L. medanensis, L. javanica & L. hebdomadis.

Thirteen of these were recovered from Chumporn province. Verbal reports received from the School of Tropical Medicine, University of Medical Sciences indicate that a high prevalence by serologic testing has been found in the human population in that area. Fourteen of the 2 rodent isolations reported above were also obtained from that area. It would appear that further investigations in that area are indicated.

In collaboration with the Department of Clinical Research, studies have been initiated on isolation of the leptospire organism from blood and urine of patients manifesting "fevers of unknown origin". One drop of freshly drawn whole blood is inoculated in a tube of Fletchers mebia and incubated at room temperature for thirty days. Specimens have been received on twenty-nine patients to date. No isolates have been recovered.

SUMMARY: Isolates from dog and rodent Kidneys in Bangkok indicate that L. bataviae is the most common isolate in animals in this municipality. L. javanica is most commonly recovered from rodents trapped in rural areas. Significant recovery rates of L. ecterohemorrhagiae were encountered in Krabi and Chumporn provinces. Recovery rates from water were highest in Ranong and Chumporn provinces in addition to the rodent isolation indicating a significant public health hazard to populations in this area. Surprisingly few isolates were recovered from the southern most provinces. Since P. pseudomallei causes early death in inoculated hamsters and a high incidence was encountered in this area, the likelihood of recovering leptospiral organisms from these specimens is minimal and prevalence may be greater than reported. One hundred fourteen isolates were recovered during the period of this report. Data collected indicate that the dog and rodent play a significant role but that at least during this period water had a less important role in the transmission of this disease in Thailand.